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(54) Title: VACCINE FOR PREVENTION AND/OR PROPHYLAXIS OF INFECTIOUS SALMON ANAEMIA IN FISH

(57) Abstract: The present invention concerns a vaccine for treatment and/or prevention of infectious salmon anaemia in fish. The invention provides for nucleic acid sequence encoding viral proteins of Infectious Salmon Anaemia Virus (ISAV) as well as the isolated (recombinant) protein, which can be used to prepare the vaccine. The invention furthermore pertains to antibodies that are reactive with said viral proteins and their use in diagnostics.

VACCINE FOR PREVENTION AND/OR PROPHYLAXIS OF INFECTIOUS SALMON ANAEMIA IN FISH.

The present invention relates to isolated viral proteins of Infectious Salmon Anaemia Virus, DNA encoding said proteins and use of said DNA or protein for diagnostic or vaccine purposes.

Infectious Salmon Anaemia (ISA) is a disease in fish caused by a virus (ISAV) that belongs to the family Orthomyxoviridae. The disease is characterised by severe anaemia, leucopenia, ascites, haemorrhagic liver necrosis and petecchia of the vicera. The gills are pale, and petecchia of the skin is also common. The spleen is dark and swollen (Speilberg et al, 1995; Veterinary Pathology, 32, pp. 466-478). The virus replicates in endothelial cells, both in blood vessels and in the heart, and in polymorphonuclear leukocytes. Budding of the virus from pillar cells in the gills has been observed, indicating that gills are probably an important portal of entrance for ISAV.

ISA was observed for the first time in Norway (Thorud et al., 1988; Bull. Eur. Ass. Fish Pathol., 8 (5), pp. 109-111) and severe outbreaks have recently been diagnosed also in Scotland, the Shetland Islands and Canada. Mortality during outbreaks varies between 10 and 100% and younger individual appear to be more susceptible than older. However, high mortality has also been observed among market size fish. Clinical outbreaks have been observed so far in Atlantic salmon, but rainbow trout and brown trout may act as carriers of the agent without developing clinical signs. Despite stamping out strategies, new outbreaks occur regularly and result in significant losses.

Control of the disease therefore has a high priority, and the present invention provides novel means to carry out such control. The present invention provides for nucleic acid sequences encoding viral proteins of the ISAV and fragments of said proteins. A first nucleic acid (SEQ ID NO 1) encodes for a protein designated Protein B of the ISAV and comprises 1083 nucleotides. This deduced amino acid sequence of Protein B is depicted in SEQ ID NO 2. A second nucleic acid sequence (SEQ ID NO 3) designated as ORF9y comprises 1737 nucleotides and encodes for another protein of ISAV. The deduced amino acid sequence of the protein encoded by ORF 9y is depicted

in SEQ ID NO 4 and comprises 578 amino acid residues. The cloning and characterisation of the nucleic acid sequence according to the invention provides for the production of proteins of the ISAV using recombinant technology (Sambrook et al., Molecular cloning: a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989). Cloning techniques and subsequent protein expression using in vitro expression systems are well known in the art. In this way, viral proteins of the ISAV can be obtained, which are substantially free from other ISAV proteins. The proteins were fond to be specific for the ISA virus, which makes these proteins very suitable for use in vaccinations and diagnostics. These isolated proteins can be used in the manufacture of vaccines to protect fish against infection with ISA virus. Said vaccines may be used as marker vaccine to distinguish vaccination from field infections with ISAV. Alternatively the nucleotide sequences encoding the ISAV proteins can be used to manufacture DNA vaccines or vector vaccines to protect fish against infection with ISAV. The nucleotide sequences and recombinant proteins of the present invention can furthermore be used for diagnostic purposes, for instance to detect the presence of the ISAV or anti-ISAV antibodies in fish. Additionally, the recombinant proteins of the present invention can be used to produce ISAV specific antibodies. These antibodies can also be used for diagnostic purposes such as the detection of ISAV in fish.

Thus, in a first aspect the present invention provides for a nucleic acid sequence comprising the nucleotide sequence depicted in SEQ ID NO 1 or SEQ ID NO 3. A nucleic acid sequence comprising a fragment of the sequences depicted in SEQ ID NO 1 or 3. For the purpose of this invention a fragment is understood to consist of 10 nucleotides or more. Also within the scope of this invention are nucleotide sequences comprising tandem arrays of the sequence depicted in SEQ ID NO 1, SEQ ID NO 3, or of fragments of these sequences. Nucleotide sequences that are complementary to the sequences depicted in SEQ ID NO 1 and SEQ ID 3 or to parts of the sequences depicted in SEQ ID NO 1 or 3 are also within the scope of the invention, as well as nucleotide sequence that hybridise with the sequence depicted in SEQ ID NO 1 or SEQ ID NO 3. The hybridisation conditions for this purpose are stringent, preferably highly stringent. According to the present invention the term "stringent" means washing conditions of 1 x SSC, 0.1% SDS at a temperature of 65°C; highly stringent conditions refer to a reduction in SSC concentration towards 0.3 x SSC.

Nucleotide sequences that hybridise with the sequence shown in SEQ ID NO 1or SEQ ID NO 3 are understood to be nucleotide sequences that have a sequence homology of at least 70%, preferably 80%, more preferably 90% with the corresponding matching part of the sequence depicted in SEQ ID NO 1 or SEQ ID NO 3. According to the present invention the sequence homology is determined by comparing the nucleotide sequence with the corresponding part of the sequence depicted in SEQ ID NO 1 or 3. The sequence homology between a nucleotide sequence of interest and the sequence in SEQ ID NO 1 or 3 can be determined via common sequence analysis programs such as BLASTN and the like. The optimal match area is determined automatically by these programs. Homologous sequences sequence from closely related ISAV strains can easily be isolated with the sequence depicted in SEQ ID NO 1 or SEQ ID NO 3 or with fragments of the sequences in SEQ ID NO 1 or 3 using routine cloning and hybridisation techniques (Sambrook et al., supra). Preferred nucleotide sequences according to the invention have a nucleotide sequence as depicted in SEQ ID NO 3, SEQ ID NO 5 or SEQ ID NO 7.

The nucleotide sequences of the invention can be used in the preparation of a DNA vaccine to vaccinate fish against ISA virus infection. DNA vaccination refers to the induction of an immune response to one or more antigens that are expressed in vivo from a gene inserted in a DNA plasmid, which has been inoculated directly into the vaccinated fish.

Thus in a second aspect of the invention there is provided for a DNA vaccine comprising a pharmaceutical acceptable carrier and a DNA plasmid in which one or more nucleotide sequences according to the invention are operably linked to a transcriptional regulatory sequence.

Preferably the nucleotide sequence to be used in said DNA plasmid is a nucleotide sequence comprising the nucleotide sequence depicted in SEQ ID NO 1 or fragments of the sequence of SEQ ID NO 1. Also suitable for use in said DNA plasmid are nucleotide sequences that are complementary to the sequence of SEQ ID NO 1 or nucleotide sequences that hybridise with the sequence of SEQ ID NO 1. The sequence homology between the nucleotide sequences that hybridise with the sequence of SEQ ID NO 1 is determined as described earlier. More preferably a DNA vaccine according to the invention comprises a nucleotide sequence comprising the nucleotide sequence od

SEQ ID NO 1 and a nucleotide sequence comprising the sequence depicted in SEQ ID NO 3. The nucleotide sequences comprising the sequence of SEQ ID NO 3 can be on the same DNA plasmid as the nucleotide sequence comprising the sequence of SEQ ID NO 1 be or can be on a separate DNA plasmid.

DNA plasmids that are suitable for use in a DNA vaccine according to the invention are conventional cloning or expression plasmids for bacterial, eukaryotic- and yeast host cells, many of which are commercially available. Well-known examples of such plasmids are pBR322 and pcDNA3 (Invitrogen). The DNA plasmids according to the invention should be able to induce protein expression of the nucleotide sequences. The DNA plasmid can comprise one or more nucleotide sequences according to the invention. In addition, the DNA plasmid can comprise other nucleotide sequences such as the immune-stimulating oligonucleotides having unmethylated CpG dinucleotides, or nucleotide sequences that code for other antigenic proteins or adjuvating cytokines.

Transcriptional regulatory sequences that are suitable for use in a DNA plasmid according to the invention comprise promoters such as the (human) cytomegalovirus immediate early promoter (Seed, B. et al., Nature 329, 840-842, 1987; Fynan, E.F. et al., PNAS 90, 11478-11482,1993; Ulmer, J.B. et al., Science 259, 1745-1748, 1993), Rous sarcoma virus LTR (RSV, Gorman, C.M. et al., PNAS 79, 6777-6781, 1982; Fynan et al., supra; Ulmer et al., supra), the MPSV LTR (Stacey et al., J. Virology 50, 725-732, 1984), SV40 immediate early promoter (Sprague J. et al., J. Virology 45, 773 ,1983), the metallothionein promoter (Brinster, R.L. et al., Nature 296, 39-42, 1982), the major late promoter of Ad2, the β-actin promoter (Tang et al., Nature 356, 152-154, 1992).The regulatory sequences may also include terminator and polyadenylation sequences. Amongst the sequences that can be used are the well-known bovine growth hormone polyadenylation sequence, the SV40 polyadenylation sequence, and the human cytomegalovirus (hCMV) terminator and polyadenylation sequences.

The DNA plasmid comprising a nucleotide sequence according to the present invention operably linked to a transcriptional regulatory sequence for use in the vaccine according to the invention can be naked or can be packaged in a delivery system. Suitable delivery systems are lipid vesicles, Iscoms, dendromers, niosomes, polysaccharide matrices, and the like. Also very suitable as delivery system are attenuated live bacteria such as Salmonella.

The nucleotide sequences according to the invention can additionally be used in the production of a vector vaccine to vaccinate fish against ISA virus infection. A vector vaccine is understood to be a vaccine in which a live, attenuated bacteria or virus has been modified so that it contains one or more heterologous nucleotide sequences inserted into its genetic material. These so called vector bacteria or -viruses are capable of coexpressing the heterologous proteins encoded by the inserted nucleotides.

Thus in a third aspect the invention provides for a vector vaccine comprising a live attenuated bacteria or virus which has been modified to comprise in their genetic material one or more of the nucleotide sequences of the present invention.

Preferably the nucleotide sequence to be used in said vector vaccine is a nucleotide sequence comprising the nucleotide sequence depicted in SEQ ID NO 1 or fragments of said nucleotide sequences. More preferebly the nucleotide sequence is a nucleotide sequence as depicted in SEQ ID NO 1, 5 or 7 respectively. Also suitable for use in said vector vaccine are nucleotide sequences that are complementary to the sequence of SEQ ID NO 1 or nucleotide sequences that hybridise with the sequence of SEQ ID NO 1. The sequence homology between the nucleotide sequences that hybridise with the sequence of SEQ ID NO 1 is determined as described earlier. More preferably a vector vaccine comprises live attenuated bacteria or -virus modified to express a nucleotide sequence comprising the sequence of SEQ ID NO 1 or fragments of the sequence of SEQ ID NO 1 as well as a nucleotide sequence comprising the sequence of SEQ ID NO 3 or fragments of the sequence of SEQ ID NO 3.

Very suitable for use as vaccine vectors are for example vaccinia virus or Semliki forest virus.

In a fourth aspect, the nucleotide sequences according to the invention can be used for the recombinant production of protein B and the ORF 9y protein, substantially free from other ISAV proteins. Thus, the invention provides for a protein encoded by a nucleic acid sequence comprising the sequence depicted in SEQ ID NO 1. More specifically the invention provides for a protein comprising an amino acid sequence as depicted in SEQ ID NO 2 and/or a derivative of said amino acid sequence. The invention provides furthermore for a protein encoded by a nucleic acid sequence comprising the sequence depicted in SEQ ID NO 3. More specifically the invention provides for a protein comprising an amino acid sequence as depicted in SEQ ID NO 4 and/or a

derivative of said amino acid sequence. Preferred proteins according to the invention are proteins having the amino acid sequence depicted in SEQ ID No 4, SEQ ID No 6 or SEQ ID NO 8.

Proteins comprising an amino acid sequence that is a derivative of the sequence depicted in SEQ ID NO 2 or SEQ ID NO 4 are understood to be proteins having alterations in their amino acid sequence with respect to the amino acid sequence depicted in SEQ ID NO 2 or SEQ ID NO 4, whereby said alterations do not affect the antigenic or immunogenic characteristics of the viral protein B or ORF 9y protein. For the purpose of this invention, antigenic characteristics of the Protein B and ORF 9y protein are understood to be the ability to induce production of antibodies that recognise and (cross)-react with the ISA virus. Immunogenic characteristics are understood to be the ability to induce an immune response in fish that protects against infection with ISA virus. The alterations that can occur in a sequence according to the present invention could for instance result from conservative amino acid substitutions, deletions, insertions, inversions or additions of (an) amino acid(s) in the overall sequence. Amino acid substitutions that are expected not to alter the immunological properties have been described. Amino acid replacements between related amino acids or replacements which have occurred frequently in evolution are, inter alia Ser/Ala, Ser/Gly, Asp/Gly, Asp/Asn, Ile/Val (see Dayhof, M.D., Atlas of protein sequence and structure, Nat. Biomed. Res. Found., Washington D.C., 1978, vol. 5, suppl. 3). Based on this information Lipman and Pearson developed a method for rapid and sensitive protein comparison (Science, 1985, vol. 227, 1435-1441) and determining the functional similarity between proteins and peptides having sequence homology. The derivative proteins according to the invention are still capable to function as the native protein or are still capable of inducing production of antibodies that recognise and (cross)-react with the ISA virus. Other derivatives according to the present invention are protein fragments that are still capable of inducing production of antibodies that recognise and (cross)-react with ISA virus or of inducing an immune response in fish that protects against infection with ISA virus. Such fragments preferably consist of 10 or more amino acid residues.

The proteins according to the invention can be prepared via standard recombinant protein expression techniques. For this purpose a nucleotide sequence

according to the invention encoding Protein B or the ORF 9y protein, a derivative protein of Protein B or the ORF 9y protein or a multimere of said proteins is inserted into an expression vector. Preferably the nucleotide sequence comprises the nucleotide sequence depicted in SEQ ID NO 1, SEQ ID NO 3, SEQ ID NO 5, SEQ ID NO 7 or one or more fragments of said depicted sequences. Also suitable are nucleotide sequences that are complementary to the sequence of SEQ ID NO 1, 3, 5 or 7. Also within the scope of the invention are nucleotide sequences of which the sequence homology with the sequence depicted in SEQ ID NO 1, SEQ ID NO 3, SEQ ID NO 5, or SEQ ID NO 7 is at least 70%, preferably 80%, more preferably 90%. The sequence homology between the nucleotide sequences that are suitable for use in the DNA plasmid is determined as described earlier.

Suitable expression vectors are, amongst others, plasmids, cosmids, viruses and YAC's (Yeast Artificial Chromosomes) which comprise the necessary control regions for replication and expression. The expression vector can be brought to expression in a host cell. Suitable host cells include but are not limited to bacteria, yeast cells, insect cells and mammalian cells. Such expression techniques are well known in the art (Sambrooke et al., Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989). Following expression, the expressed proteins can be isolated and purified from the medium.

In a further aspect the invention provides for a vaccine comprising at least a protein according to the present invention and a pharmaceutical acceptable carrier. Preferably, the protein for use in a vaccine according to the invention comprises an amino acid sequence depicted in SEQ ID NO 2 and/or a derivative of said amino acid sequence. A preferred protein for use in a vaccine according to the invention are a protein having the amino acid sequence depicted in SEQ ID NO 6 or SEQ ID NO 8. Also preferred is a vaccine comprising a mixture of a protein comprising an amino acid sequence depicted in SEQ ID NO 2 or a derivative of the sequence depicted in SEQ ID NO 2 and a protein comprising an amino acid sequence depicted in SEQ ID NO 4 and/or a derivative of the sequence depicted in SEQ ID NO 4. Surprisingly such a vaccine induced better protection in fish against infectious salmon anaemia due to a synergistic effect between said proteins. Preferably a vaccine comprises a mixture of a

protein having the amino acid sequence depicted in SEQ ID NO 6 or SEQ ID NO 8 and a protein having the amino acid sequence depicted in SEQ ID NO 4.

Vaccines according to the invention can be prepared according to techniques well known to the skilled practitioner.

Vaccines according to the invention comprise an effective amount of an immunogen according to the invention and a pharmaceutical acceptable carrier. The term "effective " as used herein is defined as the amount sufficient to induce an immune response in the target fish. An immunogen according to the invention comprises a DNA plasmid in which one or more nucleotide sequences according to the invention are operably linked to a transcriptional regulatory sequence, a vaccine vector comprising one or more nucleic acids according to the invention, or a protein according to the invention. The amount of plasmid, vector or protein will depend on the type of plasmid or vector, the route of administration, the time of administration, the species of the fish as well as age, general health and diet.

In general, a dosage of 0.01 to 1000 µg protein per kg body weight, preferably 0.5 to 500, more preferably 0.1 to 100 µg protein can be used. With respect to the DNA vaccines, generally a minimum dosage of 10 pg. up to dosages of 1000 µg have been described to be sufficient for a suitable expression of the antigens in vivo.

Pharmaceutical acceptable carriers suitable for use in a vaccine according to the invention are sterile water, saline, aqueous buffers such as PBS and the like. In addition a vaccine according to the invention may comprise other additives such as adjuvants, stabilisers, anti-oxidants and others.

Suitable adjuvants include, amongst others, aluminium hydroxide, aluminium phosphate, amphigen, tocophenols, monophosphenyl lipid A, muramyl dipeptide, oil emulsions, glucans, cytokines and saponins such as Quill A. The amount of adjuvant added depends on the nature of the adjuvant itself.

Suitable stabilisers for use in a vaccine according to the invention are for example carbohydrates including sorbitol, mannitol, starch, sucrose, dextrin, and glucose, proteins such as albumin or casein, and buffers like alkaline phosphates.

The vaccines according to the invention are administered to the fish via injection, spray, immersion or per oral. The administration protocol can be optimised in accordance with standard vaccination practice.

The nucleotide sequences and the proteins according to the invention are also suitable for use in diagnostics. The nucleotide sequences or fragments thereof can be used to detect the presence of ISAV in fish. A sample of fish infected with ISAV will comprise nucleic acid material derived from said virus, including nucleic acid sequences encoding Protein B. Suitable methods for the detection of nucleic acid sequences that are reactive with the nucleic acid sequences of the present invention include hybridisation techniques including but not limited to PCR techniques and NASBA techniques. . Nucleic acid fragments of 10 or more nucleotides are particularly suitable for use as probe in hybridisation assays or primer in amplification assays such as PCR.

The proteins according to the present invention can be used to detect the presence of anti-Protein B-antibodies in the fish. Since Protein B is characteristic for the ISA virus, the presence of antibodies against the Protein B in fish is an indication of infection with ISA virus. In general, these antibodies can be detected by an immunoassay comprising the steps of:

(i) incubating a sample suspected of containing antibodies against ISAV with Protein B antigen,

(ii) allowing the formation of antibody-antigen complex, and

(ii) detecting the presence of the antibody-antigen complex.

The design of this immunoassay may vary. For example, the immunoassay may be based upon competition or direct reaction. Furthermore, protocols may use solid supports or may use cellular material. The detection of the antibody-antigen complex may involve the use of labelled antibodies; the labels may be, for example, enzymes, fluorescent-, chemiluminescent-, radio-active- or dye molecules.

Suitable methods for the detection of antibodies reactive with a protein according to the present invention in the sample include the enzyme-linked immunosorbent assay (ELISA), immunofluorescent test (IFT) and Western blot analysis.

The proteins according to the invention can additionally be used for the production of antibodies, using the general techniques available to the practitioner in the field. Antibodies that are produced with a protein according to the invention have the advantage of specifically reacting with Protein B of ISA virus. Preferably the proteins are used to produce specific monoclonal antibodies. The obtained antibodies may be utilised in diagnostics, to detect the presence of ISAV in the fish.

Thus, in another aspect, the present invention provides for a diagnostic kit comprising a suitable means for detection and one or more nucleotide sequences according to the invention, or one or more proteins according to the invention, or antibodies obtained with said proteins, respectively.

Antibodies according to the invention can be prepared according to standard techniques. Procedures for immunising animals, e.g. mice with proteins and selection of hybridomas producing immunogen specific monoclonal antibodies are well known in the art (see for example Coligan et al. (eds), Current protocols in Immunology, 1992; Kohler and Milstein, Nature 256:495-497, 1975; Steenbakkers et al., Mol. Biol. Rep. 19:125-134, 1994). The antibody is preferably a monoclonal antibody.

Legend to figures

Figure 1 (ISA 008): Cumulative mortality after challenge with ISAV Bremnes 98 in presmolts of Atlantic Salmon (Salman salar) vaccinated with 0.2 ml vaccine suspensions or saline. 1H = vaccination with 50 µg irrelevant protein, positive control. 9Y = vaccination with 50 µg 9Y protein. 9Z = vaccination with 5µg 9Z protein. 9Y/9Z = vaccination with a mixture of 50 µg 9Y and 5µg 9Z protein. Saline = vaccination with saline, negative control.

Figure 2 (ISA 009): Cumulative mortality after challenge with ISAV Bremnes 98 in presmolts of Atlantic Salmon (Salman salar) vaccinated with 0.2 ml vaccine suspensions or saline. 9Z = vaccination with 50 µg 9Z protein. 9Z-M = vaccination with 50 µg 9Z-M protein. 9Z/9Z-M = vaccination with a mixture of 50 µg 9Z and 50 µg 9Z-M protein. Saline = vaccination with saline, negative control.

MATERIALS AND METHODS

Virus isolation and construction of cDNA library

Kidney samples were taken from Atlantic salmon (*Salmo salar L.*) during an outbreak of ISA at Bremnes (Norway) in 1998. The samples were homogenized and cleared by centrifugation before filtration (0.2 µm). The homogenates were diluted 1:100

in phosphate buffered saline and incubated for 3 h at 15 °C in cell culture flasks with mono layers of ASK (Atlantic Salmon Kidney) cells. The inoculum was then replaced by cell culture medium (L-15 supplemented with 5% FCS, 50 μg ml^{-1} gentamicin and 4 mM L- glutamine) and the cultures were incubated at 15 °C.

RNA was isolated from ISAV infected ASK cells using the Trizol reagent (Life Technologies). Cells were infected with ISAV and total RNA was isolated at days 2, 3 and 4 post infection. The RNA was pooled and 2 μg was used for cDNA synthesis with the cDNA Synthesis Kit (Stratagene). A unidirectional bacteriophage Lambda cDNA library was then constructed using the Uni-ZAP XR vector and Gigapack III Gold packaging extract (Stratagene).

Screening of bacteriophage Lambda cDNA library

SEQ ID 1 and 5 were identified by immunoscreening with a polyclonal anti ISAV rabbit sera (see below) using the picoBlue Immunoscreening Kit (Stratagene). PCR products from clones suspected to be ISAV derived were produced using vector primers and the products were sequenced. One set of internal PCR primers was constructed for each sequence, and this primer pair was employed on cDNA from ISAV infected cells and uninfected cells to determine whether the sequence was viral. The pBlueScript plasmid was then excised from ISAV positive clones using the ExAssist helper phage and the SOLR strain of *E. coli* (Stratagene). Complete sequencing was performed on the isolated plasmids. SEQ ID NO 3 was identified by negative selection. [^{32}P]dATP labeled probes were prepared against the known viral genes using the Prime-It II Random Primer Labeling Kit (Stratagene). Plaque screening was performed according to standard procedures (1), and negative clones were selected. PCR products were sequenced, and an internal primer set was constructed for those sequences not identified as known cellular genes. This primer pair was employed on cDNA from ISAV infected cells and control cells to determine whether the sequence was viral. The pBlueScript plasmid was then excised from ISAV positive clones, and complete sequencing was performed on the isolated plasmids. To obtain full-length cDNA sequences, 5' RACE was performed with the 5'RACE System, Version 2.0 (Life Technologies). RACE products were cloned into the pCR 2.1-TOPO vector using the TOPO TA Cloning Kit (Invitrogen) and sequenced as described below.

DNA sequencing and assembly

Plasmids and PCR products were sequenced using the BigDye Terminator Sequencing Kit and an ABI 377 DNA analyzer (PE Biosystems). Sequences were assembled with the Sequencher software (Gene Codes Corporation). GeneBank searches were done with BLAST (2.0).

Preparation of antisera

ISAV virions were purified on a continuous sucrose gradient as previously described (4). Polyclonal antisera were prepared by immunizing rabbits three times with six weeks intervals using approximately 50 μg of purified ISAV for each immunization. The first and second immunizations was administered subcutaneously in Freund's complete and Freund's incomplete adjuvants (Difco) respectively. The third immunization was administered intravenously in saline. The animals were bled 10 days post third immunization. Peptide antisera were prepared against the predicted proteins by Eurogentec (Belgium) using the peptides DQHRTTGDEARFTC (SEQ ID NO 9)+ CEPSVKPKTQRYGKLS (SEQ ID NO 10) for the protein depicted in SEQ ID NO 4 (designated 9Y) and MGDSRSDQSRVNPQSC (SEQ ID NO 11) + CPKMVKDFDQTSLGNT (SEQ ID NO 12) for the proteins depicted in SEQ ID No's 2, 6 and 8 (designated as 9Z-T, 9Z and 9Z-M). The peptides for each sequence were coupled to keyhole limpet haemocyanin, pooled and injected into 2 rabbits according to Eurogentec's procedures.

Northern blot analysis

Northern blotting was performed with the Northern Max Kit (Ambion). Briefly, approx. 15 μg of total RNA from ISAV infected ASK cells (3 days post infection) or from uninfected cells were separated by formaldehyde-agarose gel electrophoresis and blotted onto Hybond N nylon membranes (Amersham Pharmacia Biotech). Probes were prepared and used according to the DIG High Prime Labeling and Detection Starter Kit 2 (Boehringer Mannheim). The DIG Labeled RNA molecular weight marker 1 (Boehringer Mannheim) was run in parallel.

Baculovirus expression of 9Y and 9Z cDNA

9Y, 9Z-T and 9Z cDNA (SEQ ID NO's 3, 1 and 5, respectively) was amplified by PCR and cloned into the vector pFastBac1 (Life Technologies). 9Z-M cDNA (9Z-T cDNA with a melittin signal preceding the ORF; complete sequence depicted in SEQ ID NO 7) was also constructed. The melittin cDNA comprises nucleotide 6 to 69 of SEQ ID NO 7,and is linked by a linker sequence to the 9Z-T ORF which starts at nucleotide 84 of SEQ ID NO 7. The primers are given in Table 1.

Table 1. Primers and restriction enzymes used for cloning of different ISAV sequences into pFastBac1.

Construct	Primers	Insert cut with	Vector cut with
9Y	5'-ttggcgcgccacgagctgtttcaagat-3' (seq id no 13)	*Bss* HII *Kpn* I	*Bss* HII *Kpn* I
	5'-ggggtacccaacttattgggtactgactg-3' (seq id no 14)		
9Z	5'-ttggcgcgcaaagatggcacgattc-3' (seq id no 15)	*Bss* HII *Kpn* I	*Bss* HII *Kpn* I
	5'-ggggtaccgttgtctttctttcataatc-3' (seq id no 16)		
9Z-T and 9Z-M	5'-aggcctaatgggtgactctcgaagc-3' (seq id no 17)	*Stu* I *Kpn* I	*Stu* I *Kpn* I
	5'-ctcgacaagcttggtaccgttgtct-3' (seq id no 18)		

The constructs were transformed into TOP 10 cells (Invitrogen) and the isolated plasmids were used to transform DH10Bac competent cells (Life Technologies). Recombinant baculoviruses were constructed according to Life Technologies' recommendations.

Immunefluorescence on Sf9 cells

Sf9 cells infected with 9Y, 9Z, 9Z-M and 9Z-T recombinant baculovirus were grown in microtiter plates at 28°C. At 5 days post infection, the cells were fixed in 96% ethanol and air dried. Cells were incubated with the polyclonal rabbit antiserum for 1 hour at 37°C. After washing with PBS, fluorescein isothiocyanate conjugated goat α rabbit IgG (KPL) was added, and cells were incubated at 37°C for 1 hour. After washing, the cells were mounted with glycerol-saline and examined using an ultra violet microscope. Sf9 cells infected with a recombinant baculovirus without insert were used as negative control.

SDS-PAGE and Western blotting

Lysates of Sf9 cells or purified ISAV were separated on discontinuous SDS-PAGE gels (4% stacking and 12% separating gels) at 200 V for 40 min. Proteins were blotted onto nitrocellulose by electrophoresis at 100 V for 60 min. After blocking and washing, the membranes were incubated with peptide sera or polyclonal antisera followed by a peroxidase conjugated goat α rabbit IgG serum (BioRad).

Hemadsorption assay

Blood was sampled from Atlantic salmon and washed three times in L-15 medium (Bio Wittaker) by centrifugation (110 x g for 10 min at 4°C). The cells were then centrifuged for five min at 170 x g (4°C), and a 5 % solution was made in L-15 (100 µl cells + 9.9 ml L-15). This suspension was diluted 10 times in L-15 and added to cell monolayers (5 ml to a 25 cm^2 tissue culture flask) infected with recombinant or empty baculovirus. The cells were incubated at room temperature for 45 min, washed with L-15 to remove unattached erythrocytes and finally examined in an inverted microscope.

Vaccination experiment)

Lysate of Sf9 cells infected with 9Y, 9Z, 9Z-T and 9Z-M recombinant baculovirus were emulgated in a non-mineral oil and volumes of 0.2 ml injected into Atlantic salmon (*Salmo salar*) presmolts. The vaccine dosages of 0.2 ml contained about 50 µg of recombinant protein (with exception of the 9Z vaccine in experiment 008 (fig. 1) were 0.2 ml was found to contain 5.0 µg recombinant protein). Control fish were injected with saline or a vaccine based upon an irrelevant protein not inducing immune response

against ISAV. The fish were kept in individual tanks, and challenged 10 weeks post vaccination by intra peritoneal injection of ISAV Bremnes 98. Results are shown in figures 1 and 2.

Results and conclusions

Cloning and sequence determination of an ISAV cDNA

Immunoscreening of the bacteriophage Lambda cDNA library with the polyclonal serum prepared against whole virus identified a possible ISAV clone with an open reading frame (ORF) of 1167 bases. The clone was tentatively designated 9Z. The 9Z ORF encodes a protein with predicted molecular weight of 42.4 kDa. 9Z specific PCR primers amplified a product from ISAV infected ASK cells and not from uninfected cells. Also, a DIG labeled 9Z specific DNA probe prepared using the same primers hybridized with total RNA from ISAV infected ASK cells and not with RNA from uninfected cells. The ISAV conserved motif agcaaagat (3;5) was present immediately upstream of the ORF. Together, this clearly demonstrates that the 9Z sequence is ISAV derived and not of cellular origin. Accordingly, primers derived from and probes complementary to the 9Z sequence are useful as diagnostic tools for ISA.

Negative selection using [^{32}P]dATP labeled probes against all known ISAV genes identified 1 additional viral gene. The sequence contained an ORF of 1737 bases and was designated 9Y. The 9Y ORF encodes a protein with theoretical molecular weight 65.2 kDa. PCR primers from the 9Y sequence amplified a product from ISAV infected ASK cells and not from uninfected cells. Also, a DIG labeled DNA probe prepared using the same primers hybridized with total RNA from ISAV infected ASK cells and not with RNA from uninfected cells. Upstream of the 9Y ORF, we found the motif agctaagat. Together, this clearly demonstrates that the 9Y sequence is ISAV derived and not of cellular origin. Accordingly, primers derived from and probes complementary to the 9Y sequence are useful as diagnostic tools for ISA.

Expression of 9Y and 9Z

Expression of the 9Z cDNA in baculovirus infected Sf9 cells resulted in a protein with molecular weight approximately 43 kDa that reacted with the polyclonal antiserum prepared against whole virus and with the peptide serum in Western blotting. Lysate of

Sf9 cells infected with empty baculovirus was negative. These sera also detected a similar protein in purified virus. Accordingly, a viral protein had been expressed in the Sf9 cells. The polyclonal antiserum and the peptide serum also reacted with Sf9 cells infected with 9Z-recombinant baculovirus in IFAT, but not to cells infected with empty baculovirus. In addition, the 9Z construct reacted with the monoclonal antibody 3H6F8 in IFAT on insect cells. This monoclonal antibody has previously been shown to react with the ISAV hemagglutinin (2). Cells infected with empty baculovirus did not react with 3H6F8. A distinct hemadsorption was observed to Sf9 cells infected with 9Z-recombinant baculovirus and this hemadsorption was inhibited by addition of the polyclonal antiserum prepared against purified virus. The erythrocytes did not adsorb to cells infected with empty baculovirus. The monoclonal antibody 3H6F8 has been shown to be HA specific based on its ability to inhibit hemagglutination, neutralize virus infection in cell culture and binding to the surface of ISAV particles in immune electron microscopy reactions (2). Accordingly, it is likely that the 9Z sequence encodes the ISAV hemagglutinin, in which SEQ ID NO 1 encodes the mature protein.

Expression of the 9Z-M and 9Z-T cDNA in baculovirus infected Sf9 cells resulted in a protein with molecular weight approximately 39 kDa that reacted with the polyclonal antiserum prepared against whole virus and with the peptide serum in Western blotting. Lysate of Sf9 cells infected with baculovirus without insert was negative. Expression of the 9Y cDNA in baculovirus infected Sf9 cells resulted in a protein with molecular weight approximately 65.2 kDa.

Vaccination of salmon with 9Z reduced mortality after infection with challenge ISAV. When the dosage of 9Z protein was increased from 5 to 50 µg the mortality dropped considerably. The relatively low efficacy of vaccination with the 9Z-M protein probably results from an insufficient protein dosage. Routine experimentation will be able to establish the dosage of 9Z-M or 9Y that is sufficient to obtain an efficacious vaccine.

Surprisingly addition of the 9Y protein to a vaccine comprising 9Z or 9Z-M had a positive effect on the immune response of the fishes and consequently increased the efficacy of the vaccines based on recombinant 9Z or 9Z-M alone.

Reference List

1. 1998. Current Protocols In Molecular Biology. John Wiley & Sons, Inc.

2. **Falk, K., E. Namork, and B. H. Dannevig**. 1998. Characterization and applications of a monoclonal antibody against infectious salmon anaemia virus. Dis Aquat Org **34**:77-85.

3. **Krossoy, B., I. Hordvik, F. Nilsen, A. Nylund, and C. Endresen**. 1999. The putative polymerase sequence of infectious salmon anemia virus suggests a new genus within the Orthomyxoviridae. J Virol **73**:2136-42.

4. **Mjaaland, S., E. Rimstad, K. Falk, and B. H. Dannevig**. 1997. Genomic characterization of the virus causing infectious salmon anemia in Atlantic salmon (Salmo salar L.): an orthomyxo-like virus in a teleost. J Virol **71**:7681-6.

Claims:

1. Vaccine for protecting fish against infectious salmon anaemia characterised in that said vaccine comprises at least a protein encoded by a nucleic acid sequence comprising the nucleotide sequence depicted in SEQ ID NO 1 and/or a protein encoded by a nucleic acid sequence comprising the nucleotide sequence depicted in SEQ ID NO 3, and a pharmaceutically acceptable carrier.

2. Vaccine according to claim 1 characterised in that the vaccine comprises a protein encoded by the nucleic acid sequence depicted in SEQ ID NO 5 or 7.

3. Vaccine according to claim 1 characterised in that the vaccine comprises a protein comprising the amino acid sequence depicted in SEQ ID No 2.

4. Vaccine according to claim 3 characterised in that the vaccine comprises a protein having the amino acid sequence depicted in SEQ ID No 6 or 8 and optionally a protein having the amino acid sequence depicted in SEQ ID No 4.

5. Nucleic acid comprising the nucleic acid sequence depicted in SEQ ID NO 1 or SEQ ID NO 3.

6. Protein encoded by a nucleic acid sequence according to claim 5.

7. Protein according to claim 6 characterised in that said protein comprises an amino acid sequence as depicted in SEQ ID NO 2, SEQ ID NO 4, SEQ ID No 6 or SEQ ID No 8.

8. Pharmaceutical composition comprising a nucleic acid according to claim 5, or a protein according to claims 6 or 7, and a pharmaceutical acceptable carrier.

9. Vaccine comprising a pharmaceutically acceptable carrier and a DNA plasmid, said DNA plasmid comprising at least a nucleotide sequence according to claim 5 operably linked to a transcriptional regulatory sequence.

10. Use of a nucleic acid sequence according to claim 5 for the manufacture of a DNA vaccine for treatment and/or prophylaxis of infectious salmon anaemia in fish.

11. Use of a protein according to claim 6 or 7 for the manufacture of antibodies that are specific for infectious salmon anaemia virus.

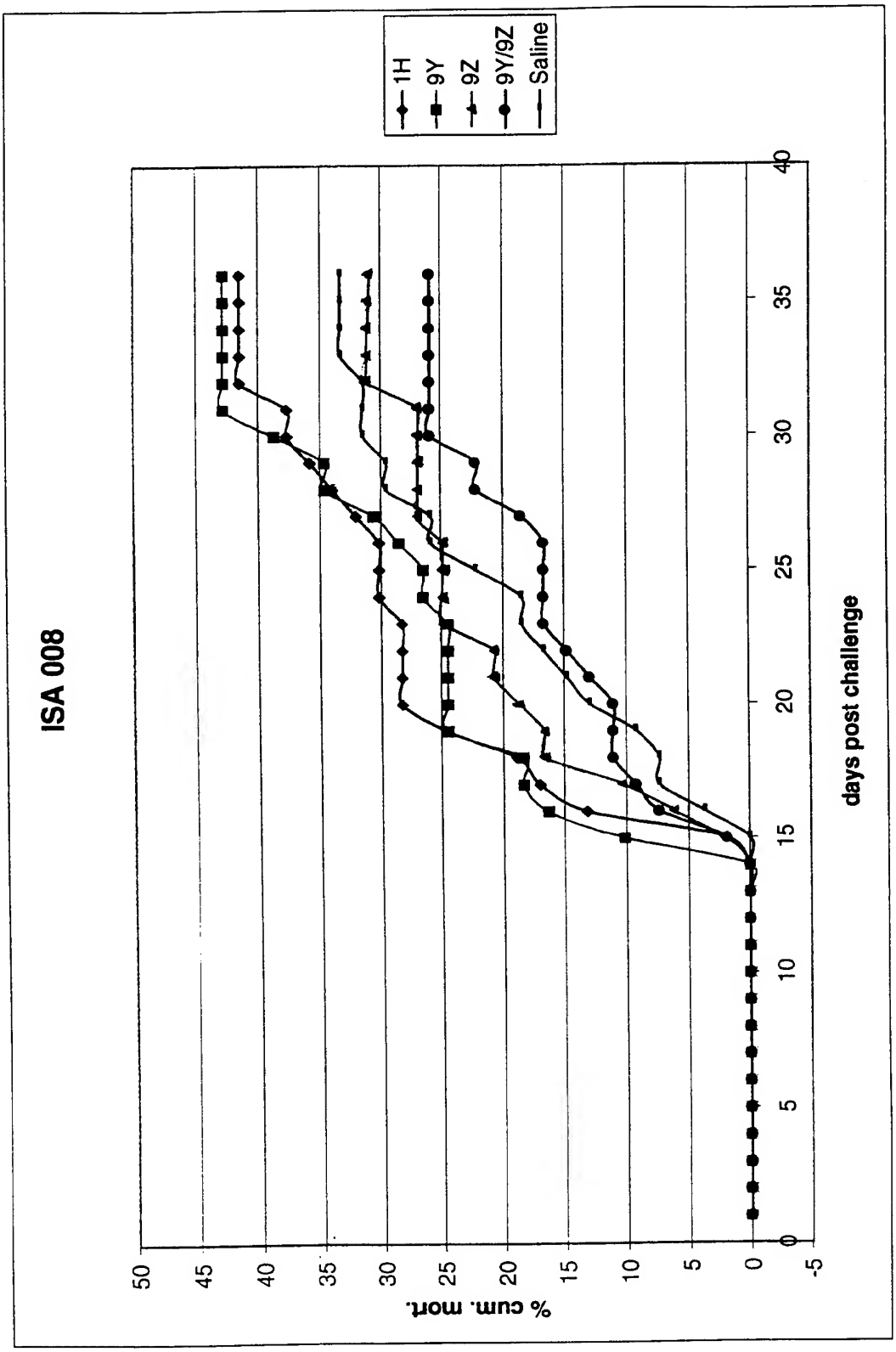
12. Antibodies raised against a protein according to claim 6 or 7.

13. A nucleic acid according to claim 5 or a protein according to claims 5 or 6 for use in diagnostics.

14. A diagnostic composition comprising a nucleic acid according to claims 1 or 2, or a protein according to claims 3-5, or antibodies that are reactive with the proteins according to claims 3-5.

15. A diagnostic kit comprising suitable detection means and a nucleic acid according to claims 1or 2, or a protein according to claims 3-5, or antibodies that are reactive with a protein according to claims 3-5.

Figure 1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1H
9Y
9Z
9Y/9Z
Saline
% cum. mort.
days post challenge
50
45
40
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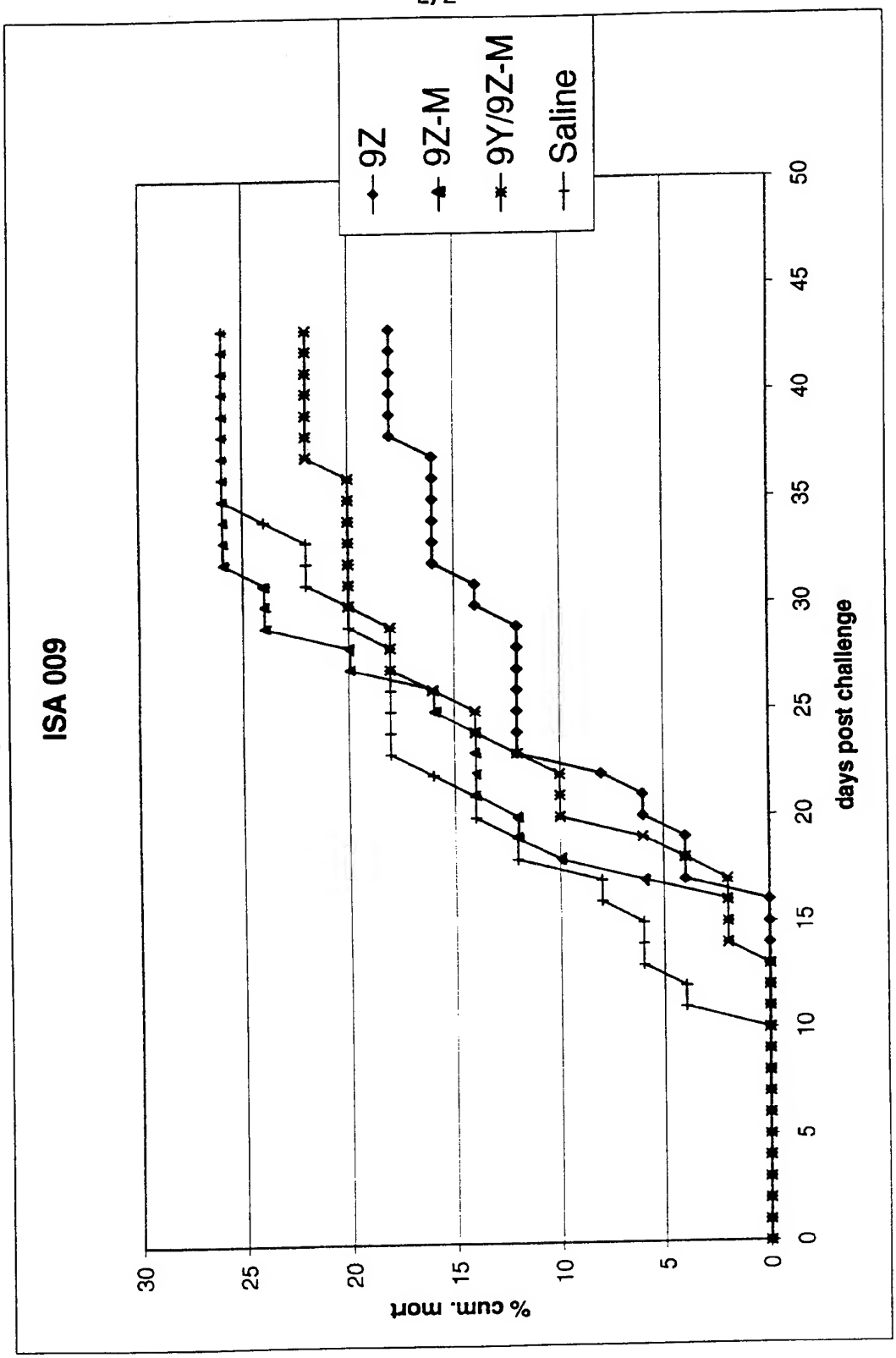
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<212> DNA
<213> infectious salmon anaemia virus

<220>
<221> CDS
<222> (1)..(1167)

<400> 5

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atg gca cga ttc ata att tta ttc cta ctg ttg gcg cct gtt tac agt   48
Met Ala Arg Phe Ile Ile Leu Phe Leu Leu Leu Ala Pro Val Tyr Ser
  1               5                  10                  15

cgt cta tgt ctt aga aac tac cct gac acc acc tgg atg ggt gac tct   96
Arg Leu Cys Leu Arg Asn Tyr Pro Asp Thr Thr Trp Met Gly Asp Ser
             20                  25                  30

cga agc gat cag tcc aga gtg aat cca cag tct ttg gat ctg gtg act   144
Arg Ser Asp Gln Ser Arg Val Asn Pro Gln Ser Leu Asp Leu Val Thr
         35                  40                  45

gag ttc aag ggg gtg ctg cag gcc aaa aac gga aat gga ctt ttg aag   192
Glu Phe Lys Gly Val Leu Gln Ala Lys Asn Gly Asn Gly Leu Leu Lys
     50                  55                  60

cag atg agt gga agg ttt cca agt gac tgg tac aca cct act aca aag   240
Gln Met Ser Gly Arg Phe Pro Ser Asp Trp Tyr Thr Pro Thr Thr Lys
 65                  70                  75                  80

tac cgg atc cta tac ttg gga acc aat gac tgc act gac gga cct act   288
Tyr Arg Ile Leu Tyr Leu Gly Thr Asn Asp Cys Thr Asp Gly Pro Thr
                 85                  90                  95

gac atg atc atc cca act tcg atg aca ctg gac aac gcg gca agg gag   336
Asp Met Ile Ile Pro Thr Ser Met Thr Leu Asp Asn Ala Ala Arg Glu
            100                 105                 110

ctg tac ctg gga gca tgc agg gga gac gtg aga gtg acg cct aca ttt   384
Leu Tyr Leu Gly Ala Cys Arg Gly Asp Val Arg Val Thr Pro Thr Phe
        115                 120                 125

gtg gga gcg gca att gtt gga ctt gtt gga cga aca gac gca att acc   432
Val Gly Ala Ala Ile Val Gly Leu Val Gly Arg Thr Asp Ala Ile Thr
    130                 135                 140

ggt ttt tcg gtg aag gtg ttg act ttc agc agc cct aca att gta gtg   480
Gly Phe Ser Val Lys Val Leu Thr Phe Ser Ser Pro Thr Ile Val Val
145                 150                 155                 160
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```
gtg gga ttg aat gga atg tcc gga atc tac aag gtc tgc att gca gca   528
Val Gly Leu Asn Gly Met Ser Gly Ile Tyr Lys Val Cys Ile Ala Ala
                165                 170                 175

aca tct ggg aat gtg gga gga gtg gca ctg atc aac gga tgc gga tat   576
Thr Ser Gly Asn Val Gly Gly Val Ala Leu Ile Asn Gly Cys Gly Tyr
            180                 185                 190

ttc aac aca cct ttg agg ttt gac aat ttc caa gga caa atc tac gtg   624
Phe Asn Thr Pro Leu Arg Phe Asp Asn Phe Gln Gly Gln Ile Tyr Val
        195                 200                 205

tca gac acc ttt gaa gtg agg gga acc aaa aac aag tgt gtt cta cta   672
Ser Asp Thr Phe Glu Val Arg Gly Thr Lys Asn Lys Cys Val Leu Leu
    210                 215                 220

aga tct tct agt gat aag cct ttg tgt tca cac atc atg agg aac gtt   720
Arg Ser Ser Ser Asp Lys Pro Leu Cys Ser His Ile Met Arg Asn Val
225                 230                 235                 240

gag cta gat gag tat gta gac aca cca aat aca ggg ggt gtt tat cct   768
Glu Leu Asp Glu Tyr Val Asp Thr Pro Asn Thr Gly Gly Val Tyr Pro
                245                 250                 255

tct gat ggt ttt gat tca cta cat ggt tca gct tcc gtt aga acg ttc   816
Ser Asp Gly Phe Asp Ser Leu His Gly Ser Ala Ser Val Arg Thr Phe
            260                 265                 270

ctc act gat gca ttg aca tgc cca gac att gac tgg agt aga att gat   864
Leu Thr Asp Ala Leu Thr Cys Pro Asp Ile Asp Trp Ser Arg Ile Asp
        275                 280                 285

gct gct tcg tgt gaa tat gac agc tgc cct aag atg gtt aaa gat ttt   912
Ala Ala Ser Cys Glu Tyr Asp Ser Cys Pro Lys Met Val Lys Asp Phe
    290                 295                 300

gac cag aca agc tta ggt aac aca gac aca ctt atc atg agg gag gta   960
Asp Gln Thr Ser Leu Gly Asn Thr Asp Thr Leu Ile Met Arg Glu Val
305                 310                 315                 320

gca ttg cac aag gag atg atc agt aaa ctt cag agg aac atc aca gat  1008
Ala Leu His Lys Glu Met Ile Ser Lys Leu Gln Arg Asn Ile Thr Asp
                325                 330                 335

gta gaa aca tct gtg ttg agc aac atc ttc att tct atg ggt gta gca  1056
Val Glu Thr Ser Val Leu Ser Asn Ile Phe Ile Ser Met Gly Val Ala
            340                 345                 350
```

```
ggt ttt ggg att gct ctg ttc cta gca ggt tgg aag gca tgt att tgg   1104
Gly Phe Gly Ile Ala Leu Phe Leu Ala Gly Trp Lys Ala Cys Ile Trp
        355                 360                 365

att gca gca ttc atg tac aag tct aga ggt aga att cca cca tcg aac   1152
Ile Ala Ala Phe Met Tyr Lys Ser Arg Gly Arg Ile Pro Pro Ser Asn
    370                 375                 380

ctg tct gtt gct tga                                           1167
Leu Ser Val Ala
385
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<210> 6
<211> 388
<212> PRT
<213> infectious salmon anaemia virus

<400> 6

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Met Ala Arg Phe Ile Ile Leu Phe Leu Leu Leu Ala Pro Val Tyr Ser
  1               5                  10                  15

Arg Leu Cys Leu Arg Asn Tyr Pro Asp Thr Thr Trp Met Gly Asp Ser
             20                  25                  30

Arg Ser Asp Gln Ser Arg Val Asn Pro Gln Ser Leu Asp Leu Val Thr
         35                  40                  45

Glu Phe Lys Gly Val Leu Gln Ala Lys Asn Gly Asn Gly Leu Leu Lys
     50                  55                  60

Gln Met Ser Gly Arg Phe Pro Ser Asp Trp Tyr Thr Pro Thr Thr Lys
 65                  70                  75                  80

Tyr Arg Ile Leu Tyr Leu Gly Thr Asn Asp Cys Thr Asp Gly Pro Thr
                 85                  90                  95

Asp Met Ile Ile Pro Thr Ser Met Thr Leu Asp Asn Ala Ala Arg Glu
            100                 105                 110

Leu Tyr Leu Gly Ala Cys Arg Gly Asp Val Arg Val Thr Pro Thr Phe
        115                 120                 125

Val Gly Ala Ala Ile Val Gly Leu Val Gly Arg Thr Asp Ala Ile Thr
    130                 135                 140

Gly Phe Ser Val Lys Val Leu Thr Phe Ser Ser Pro Thr Ile Val Val
145                 150                 155                 160
```

```
Val Gly Leu Asn Gly Met Ser Gly Ile Tyr Lys Val Cys Ile Ala Ala
                165                 170                 175

Thr Ser Gly Asn Val Gly Gly Val Ala Leu Ile Asn Gly Cys Gly Tyr
            180                 185                 190

Phe Asn Thr Pro Leu Arg Phe Asp Asn Phe Gln Gly Gln Ile Tyr Val
        195                 200                 205

Ser Asp Thr Phe Glu Val Arg Gly Thr Lys Asn Lys Cys Val Leu Leu
    210                 215                 220

Arg Ser Ser Ser Asp Lys Pro Leu Cys Ser His Ile Met Arg Asn Val
225                 230                 235                 240

Glu Leu Asp Glu Tyr Val Asp Thr Pro Asn Thr Gly Gly Val Tyr Pro
                245                 250                 255

Ser Asp Gly Phe Asp Ser Leu His Gly Ser Ala Ser Val Arg Thr Phe
            260                 265                 270

Leu Thr Asp Ala Leu Thr Cys Pro Asp Ile Asp Trp Ser Arg Ile Asp
        275                 280                 285

Ala Ala Ser Cys Glu Tyr Asp Ser Cys Pro Lys Met Val Lys Asp Phe
    290                 295                 300

Asp Gln Thr Ser Leu Gly Asn Thr Asp Thr Leu Ile Met Arg Glu Val
305                 310                 315                 320

Ala Leu His Lys Glu Met Ile Ser Lys Leu Gln Arg Asn Ile Thr Asp
                325                 330                 335

Val Glu Thr Ser Val Leu Ser Asn Ile Phe Ile Ser Met Gly Val Ala
            340                 345                 350

Gly Phe Gly Ile Ala Leu Phe Leu Ala Gly Trp Lys Ala Cys Ile Trp
        355                 360                 365

Ile Ala Ala Phe Met Tyr Lys Ser Arg Gly Arg Ile Pro Pro Ser Asn
    370                 375                 380

Leu Ser Val Ala
385
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<210> 7
<211> 1166
<212> DNA
<213> infectious salmon anaemia virus

<220>
<221> CDS
<222> (6)..(1166)

<220>
<221> sig_peptide
<222> (6)..(68)
<223> melittin signal peptide

<400> 7
gatcc atg aaa ttc tta gtc aac gtt gcc ctt gtt ttt atg gtc gta tac 50
Met Lys Phe Leu Val Asn Val Ala Leu Val Phe Met Val Val Tyr
1 5 10 15

att tct tac atc tat gcg gaa ttc aaa ggc cta atg ggt gac tct cga 98
Ile Ser Tyr Ile Tyr Ala Glu Phe Lys Gly Leu Met Gly Asp Ser Arg
20 25 30

agc gat cag tcc aga gtg aat cca cag tct ttg gat ctg gtg act gag 146
Ser Asp Gln Ser Arg Val Asn Pro Gln Ser Leu Asp Leu Val Thr Glu
35 40 45

ttc aag ggg gtg ctg cag gcc aaa aac gga aat gga ctt ttg aag cag 194
Phe Lys Gly Val Leu Gln Ala Lys Asn Gly Asn Gly Leu Leu Lys Gln
50 55 60

atg agt gga agg ttt cca agt gac tgg tac aca cct act aca aag tac 242
Met Ser Gly Arg Phe Pro Ser Asp Trp Tyr Thr Pro Thr Thr Lys Tyr
65 70 75

cgg atc cta tac ttg gga acc aat gac tgc act gac gga cct act gac 290
Arg Ile Leu Tyr Leu Gly Thr Asn Asp Cys Thr Asp Gly Pro Thr Asp
80 85 90 95

atg atc atc cca act tcg atg aca ctg gac aac gcg gca agg gag ctg 338
Met Ile Ile Pro Thr Ser Met Thr Leu Asp Asn Ala Ala Arg Glu Leu
100 105 110

tac ctg gga gca tgc agg gga gac gtg aga gtg acg cct aca ttt gtg 386
Tyr Leu Gly Ala Cys Arg Gly Asp Val Arg Val Thr Pro Thr Phe Val
115 120 125

gga gcg gca att gtt gga ctt gtt gga cga aca gac gca att acc ggt 434

```
Gly Ala Ala Ile Val Gly Leu Val Gly Arg Thr Asp Ala Ile Thr Gly
        130                 135                 140

ttt tcg gtg aag gtg ttg act ttc agc agc cct aca att gta gtg gtg   482
Phe Ser Val Lys Val Leu Thr Phe Ser Ser Pro Thr Ile Val Val Val
    145                 150                 155

gga ttg aat gga atg tcc gga atc tac aag gtc tgc att gca gca aca   530
Gly Leu Asn Gly Met Ser Gly Ile Tyr Lys Val Cys Ile Ala Ala Thr
160                 165                 170                 175

tct ggg aat gtg gga gga gtg gca ctg atc aac gga tgc gga tat ttc   578
Ser Gly Asn Val Gly Gly Val Ala Leu Ile Asn Gly Cys Gly Tyr Phe
                180                 185                 190

aac aca cct ttg agg ttt gac aat ttc caa gga caa atc tac gtg tca   626
Asn Thr Pro Leu Arg Phe Asp Asn Phe Gln Gly Gln Ile Tyr Val Ser
            195                 200                 205

gac acc ttt gaa gtg agg gga acc aaa aac aag tgt gtt cta cta aga   674
Asp Thr Phe Glu Val Arg Gly Thr Lys Asn Lys Cys Val Leu Leu Arg
        210                 215                 220

tct tct agt gat aag cct ttg tgt tca cac atc atg agg aac gtt gag   722
Ser Ser Ser Asp Lys Pro Leu Cys Ser His Ile Met Arg Asn Val Glu
    225                 230                 235

cta gat gag tat gta gac aca cca aat aca ggg ggt gtt tat cct tct   770
Leu Asp Glu Tyr Val Asp Thr Pro Asn Thr Gly Gly Val Tyr Pro Ser
240                 245                 250                 255

gat ggt ttt gat tca cta cat ggt tca gct tcc gtt aga acg ttc ctc   818
Asp Gly Phe Asp Ser Leu His Gly Ser Ala Ser Val Arg Thr Phe Leu
                260                 265                 270

act gat gca ttg aca tgc cca gac att gac tgg agt aga att gat gct   866
Thr Asp Ala Leu Thr Cys Pro Asp Ile Asp Trp Ser Arg Ile Asp Ala
            275                 280                 285

gct tcg tgt gaa tat gac agc tgc cct aag atg gtt aaa gat ttt gac   914
Ala Ser Cys Glu Tyr Asp Ser Cys Pro Lys Met Val Lys Asp Phe Asp
        290                 295                 300

cag aca agc tta ggt aac aca gac aca ctt atc atg agg gag gta gca   962
Gln Thr Ser Leu Gly Asn Thr Asp Thr Leu Ile Met Arg Glu Val Ala
    305                 310                 315

ttg cac aag gag atg atc agt aaa ctt cag agg aac atc aca gat gta  1010
```

```
Leu His Lys Glu Met Ile Ser Lys Leu Gln Arg Asn Ile Thr Asp Val
320                 325                 330                 335

gaa aca tct gtg ttg agc aac atc ttc att tct atg ggt gta gca ggt   1058
Glu Thr Ser Val Leu Ser Asn Ile Phe Ile Ser Met Gly Val Ala Gly
                340                 345                 350

ttt ggg att gct ctg ttc cta gca ggt tgg aag gca tgt att tgg att   1106
Phe Gly Ile Ala Leu Phe Leu Ala Gly Trp Lys Ala Cys Ile Trp Ile
            355                 360                 365

gca gca ttc atg tac aag tct aga ggt aga att cca cca tcg aac ctg   1154
Ala Ala Phe Met Tyr Lys Ser Arg Gly Arg Ile Pro Pro Ser Asn Leu
        370                 375                 380

tct gtt gct tga                                                  1166
Ser Val Ala
    385
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<210> 8
<211> 386
<212> PRT
<213> infectious salmon anaemia virus

<400> 8

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Met Lys Phe Leu Val Asn Val Ala Leu Val Phe Met Val Val Tyr Ile
  1               5                  10                  15

Ser Tyr Ile Tyr Ala Glu Phe Lys Gly Leu Met Gly Asp Ser Arg Ser
             20                  25                  30

Asp Gln Ser Arg Val Asn Pro Gln Ser Leu Asp Leu Val Thr Glu Phe
         35                  40                  45

Lys Gly Val Leu Gln Ala Lys Asn Gly Asn Gly Leu Leu Lys Gln Met
     50                  55                  60

Ser Gly Arg Phe Pro Ser Asp Trp Tyr Thr Pro Thr Thr Lys Tyr Arg
 65                  70                  75                  80

Ile Leu Tyr Leu Gly Thr Asn Asp Cys Thr Asp Gly Pro Thr Asp Met
                 85                  90                  95

Ile Ile Pro Thr Ser Met Thr Leu Asp Asn Ala Ala Arg Glu Leu Tyr
            100                 105                 110

Leu Gly Ala Cys Arg Gly Asp Val Arg Val Thr Pro Thr Phe Val Gly
```

```
        115                 120                 125

Ala Ala Ile Val Gly Leu Val Gly Arg Thr Asp Ala Ile Thr Gly Phe
    130                 135                 140

Ser Val Lys Val Leu Thr Phe Ser Ser Pro Thr Ile Val Val Val Gly
145                 150                 155                 160

Leu Asn Gly Met Ser Gly Ile Tyr Lys Val Cys Ile Ala Ala Thr Ser
                165                 170                 175

Gly Asn Val Gly Gly Val Ala Leu Ile Asn Gly Cys Gly Tyr Phe Asn
            180                 185                 190

Thr Pro Leu Arg Phe Asp Asn Phe Gln Gly Gln Ile Tyr Val Ser Asp
        195                 200                 205

Thr Phe Glu Val Arg Gly Thr Lys Asn Lys Cys Val Leu Leu Arg Ser
    210                 215                 220

Ser Ser Asp Lys Pro Leu Cys Ser His Ile Met Arg Asn Val Glu Leu
225                 230                 235                 240

Asp Glu Tyr Val Asp Thr Pro Asn Thr Gly Gly Val Tyr Pro Ser Asp
                245                 250                 255

Gly Phe Asp Ser Leu His Gly Ser Ala Ser Val Arg Thr Phe Leu Thr
            260                 265                 270

Asp Ala Leu Thr Cys Pro Asp Ile Asp Trp Ser Arg Ile Asp Ala Ala
        275                 280                 285

Ser Cys Glu Tyr Asp Ser Cys Pro Lys Met Val Lys Asp Phe Asp Gln
    290                 295                 300

Thr Ser Leu Gly Asn Thr Asp Thr Leu Ile Met Arg Glu Val Ala Leu
305                 310                 315                 320

His Lys Glu Met Ile Ser Lys Leu Gln Arg Asn Ile Thr Asp Val Glu
                325                 330                 335

Thr Ser Val Leu Ser Asn Ile Phe Ile Ser Met Gly Val Ala Gly Phe
            340                 345                 350

Gly Ile Ala Leu Phe Leu Ala Gly Trp Lys Ala Cys Ile Trp Ile Ala
        355                 360                 365

Ala Phe Met Tyr Lys Ser Arg Gly Arg Ile Pro Pro Ser Asn Leu Ser
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370 375 380

Val Ala
385

<210> 9
<211> 14
<212> PRT
<213> infectious salmon anaemia virus

<400> 9
Asp Gln His Arg Thr Thr Gly Asp Glu Ala Arg Phe Thr Cys
1 5 10

<210> 10
<211> 16
<212> PRT
<213> infectious salmon anaemia virus

<400> 10
Cys Glu Pro Ser Val Lys Pro Lys Thr Gln Arg Tyr Gly Lys Leu Ser
1 5 10 15

<210> 11
<211> 16
<212> PRT
<213> infectious salmon anaemia virus

<400> 11
Met Gly Asp Ser Arg Ser Asp Gln Ser Arg Val Asn Pro Gln Ser Cys
1 5 10 15

<210> 12
<211> 16
<212> PRT
<213> infectious salmon anaemia virus

<400> 12
Cys Pro Lys Met Val Lys Asp Phe Asp Gln Thr Ser Leu Gly Asn Thr
1 5 10 15

<210> 13
<211> 27
<212> DNA
<213> infectious salmon anaemia virus

<400> 13
ttggcgcgcc acgagctgtt tcaagat 27

<210> 14
<211> 29
<212> DNA
<213> infectious salmon anaemia virus

<400> 14
ggggtaccca acttattggg tactgactg 29

<210> 15
<211> 25
<212> DNA
<213> infectious salmon anaemia virus

<400> 15
ttggcgcgca aagatggcac gattc 25

<210> 16
<211> 28
<212> DNA
<213> infectious salmon anaemia virus

<400> 16
ggggtaccgt tgtctttctt tcataatc 28

<210> 17
<211> 25
<212> DNA
<213> infectious salmon anaemia virus

<400> 17
aggcctaatg ggtgactctc gaagc 25

<210> 18
<211> 25
<212> DNA
<213> infectious salmon anaemia virus

<400> 18
ctcgacaagc ttggtaccgt tgtct 25